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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/922,549	08/03/2001	Jeffrey C. Rapp	AVI 013N	1388

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EXAMINER
MCGILLEM, LAURA L

ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 07/06/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/922,549	RAPP, JEFFREY C.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 April 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *175-216* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *175-216* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *03 November 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

It is noted that claims 1-174 have been canceled and claims 175-216 have been added in the amendment field 4/7/2006. Claims 175-216 are under examination.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 197-206 are rejected under 35 U.S.C. 101 because they are directed to non-statutory subject matter. Claim 197 is drawn to a DNA molecule integrated into cellular genome. The cells can be in a human and claims reading on *in vivo* human tissue are non-statutory because the claims read on part of a living human being *in situ*.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 175-216 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants claim an expression vector integrated into a cellular genome comprising a gene expression controlling region comprising a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:37 or hybridizes to the complement of the nucleotide sequence of SEQ ID NO:67 in the presence of 1.0 M Na ion at 60°C. Applicants also claim a tubular gland cell containing said expression vector Applicants claim a DNA molecule integrated into a cellular genome comprising a gene expression controlling region operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the gene expression controlling region comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67 or hybridizes to the complement of the nucleotide sequence of SEQ ID NO:67 in the presence of 1.0M Na ion at 60°C. Applicants also claim a tubular gland cell containing said DNA molecule.

The written description requirement for a genus may be satisfied by sufficient description of a representative number of species by actual reduction to practice or by disclosure of relevant identifying characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that applicant was in possession of the claimed invention. As the instant claims are written, they are drawn to "a [any] nucleic acid sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67", which encompasses nucleic acids that comprise the full-length sequence of SEQ ID NO:67, any smaller portion of SEQ ID NO:67 and a wide variety of variants of SEQ ID NO:67. Further, the specification discloses said hybridization conditions as "stringent" for long

probes, as compared to "moderate stringency" or "high stringency" (see Patent Application Pub. paragraph 0060). Therefore, the claims encompass an extremely large genus of nucleotide sequences that can hybridize to SEQ ID NO:67 under relatively low stringency hybridization conditions.

In the instant case, the specification discloses one sequence (SEQ ID NO:67) for the claimed gene expression controlling region. There is no description of how the structure of the disclosed SEQ ID NO:67 relates to the structure of gene expression controlling regions that might be significantly shorter fragments of SEQ ID NO:67. The genus would be expected to have divergent functional properties as changes in length of the nucleotide sequence can have significant effects on the structure and properties of expression controlling sequences. The applicant does not provide an indication of how the sequence of SEQ ID NO:67 is representative of the multitude of sequences that are dinucleotides or larger oligonucleotides that hybridize to SEQ ID NO:67 or to the complement of SEQ ID NO:67 under relatively low stringency conditions and would be able to function as an gene expression controlling region for a heterologous polypeptide. There is no description any of the extremely large genus of shorter nucleotide fragments of SEQ ID NO:67 that would be able to perform the function of the full nucleotide sequence of SEQ ID NO:67 to control gene expression. According to these facts, one of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only one member of this genus is not representative of the variant of the genus and is insufficient to support them.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 185 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 185 recites the limitation "the isolated DNA molecule of claim 184". There is insufficient antecedent basis for this limitation in the claim. Claim 184 is drawn to an expression vector and does not specifically recite an isolated DNA molecule. Therefore, it is not clear what isolated DNA molecule is intended as a limitation of the claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

It should be noted that independent claims 175, 186, 197 and 207 are drawn to "a nucleic acid sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67", which encompasses nucleic acids that comprise the full-length sequence of SEQ ID NO:67 or any portion of SEQ ID NO:67 including a dinucleotide or larger oligonucleotide. The specification discloses said hybridization conditions as "stringent" for long probes, as compared to "moderate stringency" or "high stringency" (see Patent Application Pub. paragraph 0060). At the claimed hybridization conditions and absent

evidence to the contrary, the skilled artisan would reasonably expect nucleotide sequences which are 87-100% identical to the claimed SEQ ID NO:67 to be able to hybridize to SEQ ID NO:67.

Claims 175-177, 182-185, 197-199 and 204-206 are rejected under 35 U.S.C. 103(a) as being unpatentable over Phi-Van and Stratling (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) evidenced by Phi-Van and Stratling (1988 of record) and as evidenced by the online BioTech Life Science Dictionary (chloramphenicol acetyl transferase entry). This is a NEW rejection.

Applicant claims an expression vector comprising a gene expression controlling region comprising a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67 or hybridizes to the complement of the nucleotide sequence of SEQ ID NO:67, each hybridization in the presence of 1.0 M Na ion at a temperature of 60°C, wherein the expression vector is integrated into a cellular genome. Applicant further claims said expression vector wherein it comprises a 5' matrix attachment region, a transcription enhancer or a polyadenylation signal sequence derived from the SV40 virus and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest. It is noted that the disclosure does not specifically define "protein of pharmaceutical interest" which allows a broad interpretation of what proteins are of pharmaceutical interest.

Phi-Van et al teach a nucleotide sequence (GenBank Accession No. X98408) from the chicken lysozyme gene transcription enhancer region that is 96% identical to nucleotides 1-237 and 261-1564 of SEQ ID NO:67. Phi-Van et al teach that a chicken lysozyme gene promoter comprising a **5' matrix attachment** region was operably linked to a chloramphenicol acetyltransferase (CAT) reporter gene (i.e. a heterologous polypeptide) in a plasmid including a **SV40 polyadenylation sequence** (see page 10736, left column, last paragraph, and right column, Figure 1, in particular). The on-line BioTech Life Science Dictionary defines Chloramphenicol acetyl transferase (CAT) gene as "a gene which codes for the CAT enzyme which helps transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT, as an enzyme active with an antibiotic, is a protein of pharmaceutical interest. Phi-Van teaches that the expression plasmids were transfected into cultured fibroblasts for CAT expression assays (see page 10736, right column, last paragraph, for example).

Phi-Van et al do not teach that the expression vector is integrated into a cellular genome.

Sippel and Stief teach that expression of a gene of interest can be increased when an enhancer element, promoter element and gene of interest are flanked with attachment (A) elements and integrated into the genomic of eukaryotic cells independently of the chromosomal position of the gene (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of A-elements are taken from the chromatin domain (matrix attachment regions) of the chicken lysozyme gene as cited in Phi Van and Stratling (1988)(column 1, lines 54-67, for example). Sippel and

Stief exemplify that a chicken lysozyme promoter, enhancer and reporter gene encoding CAT were cloned into a plasmid and transfected into promacrophage cells (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of the promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example).

It would have been obvious to one of ordinary skill in the art to combine the plasmid construct comprising a lysozyme gene enhancer and promoter comprising a 5' matrix attachment region operably linked to a CAT reporter gene and including a SV40 polyadenylation sequence as taught by Phi-Van et al (1996) with the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT expression is increased when a construct containing lysozyme matrix attachment regions linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme promoter CAT construct into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175-176, 184-185, 197-198 and 206 are rejected under 35 U.S.C. 103(a) as being unpatentable over von Kries et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (chloramphenicol acetyl transferase entry). This is a NEW rejection.

Applicant claims an expression vector comprising a gene expression controlling region comprising a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67 or hybridizes to the complement of the nucleotide sequence of SEQ ID NO:67, each hybridization in the presence of 1.0 M Na ion at a temperature of 60°C, where in the expression vector is integrated into a cellular genome. Applicant further claims said expression vector wherein it comprises a 5' matrix attachment region or an intrinsically curved region of DNA, a transcription enhancer and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide.

von Kries et al teach a nucleotide sequence (GenBank Accession No. X52989) from the chicken lysozyme gene 5' matrix attachment region that is 98% identical to nucleotides 2011-2671 of SEQ ID NO:67 and strongly curved. von Kries et al teach that an isolated DNA sequence was cloned into a plasmid vector for sequencing (see page 3881, right column, 1st paragraph and page 3882, left column, 2nd paragraph, for example), which reads on an isolated DNA molecule and an expression vector comprising a nucleotide sequence or an **intrinsically curved region** of DNA that hybridizes to the nucleotide sequence of SEQ ID NO:67.

von Kries et al do not teach that the expression vector comprising an intrinsically curved lysozyme gene 5' matrix attachment region is integrated into a cellular genome.

Sippel and Stief teach that expression of a gene of interest can be increased when an enhancer element, promoter element and gene of interest are flanked with A-elements and integrated into the genomic of eukaryotic cells independently of the chromosomal position of the gene (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of A-elements are taken from the matrix attachment regions of the chicken lysozyme gene (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter, enhancer and reporter gene encoding CAT (i.e. heterologous polypeptide) were cloned into a plasmid and transfected into promacrophage cells (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of the promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example). The on-line BioTech Life Science Dictionary defines the CAT gene as "a gene which codes for the CAT enzyme which helps transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT, as an enzyme active with an antibiotic, is a protein of pharmaceutical interest.

It would have been obvious to one of ordinary skill in the art to combine the plasmid construct comprising a **curved** lysozyme gene 5' matrix attachment region as taught by von Kries et al with the chromosomally integrated expression plasmid for CAT taught by Sippel and Stief, because Sippel and Stief teach that gene expression is

increased when a construct containing lysozyme promoter elements such as matrix attachment regions linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating a plasmid expression vector containing a **curved** lysozyme gene 5' matrix attachment region into the host cell genome and expressing CAT since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175, 177, 184-185, 197, 199 and 206 are rejected under 35 U.S.C. 103(a) as being unpatentable over Grewal et al (of record) as evidenced by the online BioTech Life Science Dictionary (CAT entry) in view of Sippel and Stief (U.S. Patent 5,731,178). This is a NEW rejection.

Applicant claims an expression vector as described herein above, wherein the expression vector is integrated into a cellular genome. Applicant further claims said expression vector wherein it comprises a transcription enhancer and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Grewal et al teach a nucleotide sequence from the chicken lysozyme gene enhancer region that is located 6.1 kb upstream of the lysozyme transcription initiation site and which is 100% identical to nucleotides 5848-5934 of SEQ ID NO:67 (see page 2344, Figure 4 in particular). The -6.1kb **enhancer element** was isolated and cloned into a plasmid comprising reporter gene such as CAT (see page 2340, left column). Grewal et al teach that the expression vector was transfected into several cell types and that the –6.1 kb lysozyme gene enhancer plays a central role in the activation of lysozyme expression (see page 2340, right column, last paragraph and page 2347, right column, for example). The on-line BioTech Life Science Dictionary defines CAT gene as "a gene which codes for the CAT enzyme which helps transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT, as an enzyme active with an antibiotic, is a protein of pharmaceutical interest.

Grewal et al do not teach that the expression vector comprising a -6.1kb enhancer element was integrated into a cellular genome.

Sippel and Stief teach that expression of a gene of interest can be increased when an **enhancer element**, promoter element and gene of interest are flanked with A-elements and integrated into the genomic of eukaryotic cells independently of the chromosomal position of the gene (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of A-elements are taken from the chicken lysozyme gene (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter, **enhancer** and reporter gene encoding CAT were cloned into a plasmid and transfected into promacrophage cells (see column 2, lines 20-25 and

52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of the promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example).

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme enhancer as taught by Grewal et al with the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter and transcription enhancer elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and enhancers into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175, 181, 184-185, 197, 203 and 206 are rejected under 35 U.S.C. 103(a) as being unpatentable over Renkawitz et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (CAT entry). This is a NEW rejection.

Applicant claims an expression vector described *supra* and a DNA molecule integrated into a cellular genome, wherein they comprise a proximal lysozyme promoter and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Renkawitz et al teach a nucleotide sequence (GenBank Accession No. M12532) from the chicken lysozyme gene promoter region that is 100% identical to nucleotides 11563-11877 of SEQ ID NO:67 and is a proximal lysozyme promoter, which has been cloned into a recombinant plasmid and injected into chicken oviduct cells (see page 504, left column, Figure 1 and page 509, left column, 2nd and 3rd paragraph). Renkawitz et al teach that the promoter is linked to a gene for T antigen (i.e. a heterologous polypeptide) (see page 504, left column, 2nd paragraph, bridging to right column, 1st paragraph).

Renkawitz et al do not teach that the plasmid comprising the proximal lysozyme promoter is integrated into the genome.

As discussed above, Sippel and Stief teach that expression of a gene of interest can be increased when a promoter element and gene of interest are flanked with matrix attachment elements and integrated into the genome of eukaryotic cells (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of matrix attachment regions are taken from the chicken lysozyme promoter (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter and reporter gene encoding CAT were cloned into a plasmid and transfected into

promacrophage cells (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of the promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example). Chloramphenicol acetyl transferase is defined in the on-line BioTech Life Science Dictionary as "a gene which codes for the CAT enzyme which helps to transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT, as an enzyme active with an antibiotic, is a protein of pharmaceutical interest.

It would have been obvious to one of ordinary skill in the art to use the expression construct comprising a lysozyme proximal promoter as taught by Renkawitz et al in the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme matrix attachment regions, promoters and enhancers linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements, such as proximal promoter regions by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and enhancers into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that

said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175, 177-178, 184-185, 197, 199-200 and 206 are rejected under 35 U.S.C. 103(a) as being unpatentable over Steiner et al (of record) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and in view of Sippel and Stief (U.S. Patent 5,731,178). This is a NEW rejection.

Applicant claims an expression vector described *supra* and a DNA molecule integrated into a cellular genome, wherein they comprise a transcription enhancer, or wherein they comprise a negative regulatory element and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is of pharmaceutical interest.

Steiner et al teach a nucleotide sequence (E-0.2kb) (GenBank Accession No. X05461) from the chicken lysozyme gene transcription enhancer region that is 98% identical to nucleotides 9160-9329 of SEQ ID NO:67 (see page 4174, Figure 8). Steiner et al also teach a nucleotide sequence (GenBank Accession No. X05463) from the chicken lysozyme gene **transcription enhancer** region that is 98% identical to nucleotides 9325-9626 of SEQ ID NO:67 and a **negative regulatory element** (see page 4174, Figure 8). Steiner et al teach that the transcription enhancer region and negative regulatory element were isolated and cloned into plasmids containing the CAT gene (see page 4164, for example). Steiner et al teach that the plasmids were transfected into chicken fibroblast cells for CAT expression assays (see page 4166, last

paragraph; page 4166, 2nd and 3rd paragraph, for example). Chloramphenicol acetyl transferase is defined in the on-line BioTech Life Science Dictionary as "a gene which codes for the CAT enzyme which helps to transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT is a protein of pharmaceutical interest.

Steiner et al do not teach that the expression vector is integrated into a cellular genome.

As discussed previously, Sippel and Stief teach that expression of a gene of interest can be increased when a promoter element and gene of interest are flanked with matrix attachment elements and enhancers and integrated into the genome of eukaryotic cells (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of matrix attachment regions and enhancers are taken from the chicken lysozyme promoter (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter and reporter gene encoding CAT were cloned into a plasmid and transfected into promacrophage cells (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example).

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme enhancer and negative regulatory element driving CAT expression as taught by Steiner et al with the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT gene

expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters, enhancers and negative regulatory elements into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175, 179, 184-185, 197, 201 and 206 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hecht et al (of record) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and in view of Sippel and Stief (U.S. Patent 5,731,178). This is a NEW rejection.

Applicant claims an expression vector described above and a DNA molecule integrated into a cellular genome, wherein they comprise at least one hormone responsive element and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Hecht et al teach a nucleotide sequence (GenBank Accession No. X12509) from the chicken lysozyme gene transcription enhancer region that is 99% identical to nucleotides 9621-9666, 9680-10060 of SEQ ID NO:67 (see page 2070, Figure 6). Hecht et al teaches a DNA sequence which stimulates transcription in a **hormone dependent manner** which has been operably linked to a CAT gene in a vector for transfection into a hormone receptor containing cell line (see page 2063, right column, 2nd and 3rd paragraph, for example). Chloramphenicol acetyl transferase is defined in the on-line BioTech Life Science Dictionary as "a gene which codes for the CAT enzyme which helps to transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT is a protein of pharmaceutical interest.

Hecht et al do not teach that the expression vector comprising a hormone responsive element is integrated into a genome.

Sippel and Stief teach that expression of a gene of interest can be increased when a promoter and enhancer element and gene of interest are flanked with matrix attachment elements and integrated into the genome of eukaryotic cells (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of matrix attachment regions are taken from the chicken lysozyme gene (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter and reporter gene encoding CAT were cloned into a plasmid (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example).

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising lysozyme gene transcription enhancer region comprising a hormone response element to drive CAT expression as taught by Steiner et al with the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and enhancers into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 175, 180, 184-185, 197, 202 and 204 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stumph et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (CAT entry). This is a NEW rejection.

Applicant claims an expression vector (described above) and a DNA molecule integrated into a cellular genome, wherein they comprise an avian CR1 repeat element

and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Stumph et al teach a nucleotide sequence (GenBank Accession No. K02907) that is a CR1 chicken enhancer repeat element that is 87% identical to nucleotides 10926-11193 of SEQ ID NO:67(see page 6669, Figure 2, for example). Stumph et al teach that CR1 sequences were cloned for sequencing into a plasmid vector (see page 6667, right column, 2nd and 3rd paragraph, for example).

Stumph et al do not teach an expression vector integrated into a cellular genome.

Sippel and Stief teach that expression of a gene of interest can be increased when a promoter and enhancer element and gene of interest are flanked with matrix attachment elements and integrated into the genome of eukaryotic cells (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of matrix attachment regions are taken from the chicken lysozyme gene promoter (column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter and reporter gene encoding CAT were cloned into a plasmid (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of promacrophage cells and that CAT activity was increased as compared to non-genomically integrated CAT constructs (see column 3, lines 3-30, for example). Chloramphenicol acetyl transferase is defined in the on-line BioTech Life Science Dictionary as "a gene which codes for the CAT enzyme which helps to transfer an acetyl

group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT is a protein of pharmaceutical interest.

It would have been obvious to one of ordinary skill in the art to combine a CR1 chicken enhancer repeat element to drive gene expression as taught by Steiner et al with the expression vector and method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme promoter elements and enhancers linked to a CAT gene is integrated into a cellular genome. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into host cell genomes. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and enhancers into the host cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Phi-Van and Stratling (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) evidenced by Phi-Van and Stratling (1988 of record) and evidenced by the online BioTech Life Science Dictionary (CAT entry) and further in view of Ivarie et al (U.S. Patent No. 6,730,822). This is a NEW rejection.

Claims 186-188, 193-196, 207-209 and 214-216 are rejected under 35 U.S.C. 103(a) as being obvious over Phi-Van and Stratling (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) evidenced by Phi-Van and Stratling (1988 of record) and evidenced by the online BioTech Life Science Dictionary (CAT entry) and further in view of Ivarie et al (U.S. Patent No. 6,730,822). This is a NEW rejection..

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell containing an expression vector comprising a gene expression controlling region comprising a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67 or hybridizes to the complement of the

nucleotide sequence of SEQ ID NO:67, each hybridization in the presence of 1.0 M Na ion at a temperature of 60°C. Applicant also claims a tubular gland cell containing an DNA molecule comprising a gene expression controlling region operably linked to a nucleic acid molecule encoding a heterologous polypeptide wherein the a gene expression controlling region comprising a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:67 or hybridizes to the complement of the nucleotide sequence of SEQ ID NO:67, at the conditions above. Applicant further claims said tubular gland cell wherein it comprises a 5' matrix attachment region, a transcription enhancer or a polyadenylation signal sequence derived from the SV40 virus. Applicant claims a tubular gland cell comprising a heterologous polypeptide is a protein of pharmaceutical interest.

Phi-Van et al teach a nucleotide sequence (GenBank Accession No. X98408) from the chicken lysozyme gene transcription enhancer region that is 96% identical to nucleotides 1-237 and 261-1564 of SEQ ID NO:67. Phi-Van et al teach that a chicken lysozyme gene promoter comprising a 5' matrix attachment region was operably linked to a CAT reporter gene (i.e. a heterologous polypeptide) in a plasmid including a SV40 polyadenylation sequence (see page 10736, left column, last paragraph, and right column, Figure 1, in particular). Phi-Van teaches that the expression plasmids were transfected into cultured fibroblasts for CAT expression assays (see page 10736, right column, last paragraph, for example). Chloramphenicol acetyl transferase is defined in the on-line BioTech Life Science Dictionary as "a gene which codes for the CAT

enzyme which helps to transfer an acetyl group to chloramphenicol, an antibiotic". Absent evidence to the contrary, CAT is a protein of pharmaceutical interest.

Phi-Van et al do not teach that the expression vector is integrated into a cellular genome. Phi-Van et al do not teach a tubular gland cell containing the claimed expression vector or DNA molecule.

Sippel and Stief teach that expression of a gene of interest can be increased when an enhancer element, promoter element and gene of interest are flanked with A-elements and integrated into the genomic of eukaryotic cells independently of the chromosomal position of the gene (see column 1, lines 11-20, for example). Sippel and Stief teach that preferred embodiments of A-elements are taken from the chromatin domain (matrix attachment regions) of the chicken lysozyme gene as cited in Phi Van and Stratling (1988)(column 1, lines 54-67, for example). Sippel and Stief exemplify that a chicken lysozyme promoter, enhancer and reporter gene encoding CAT were cloned into a plasmid and transfected into promacrophage cells (see column 2, lines 20-25 and 52-60, for example). Sippel and Stief teach that the construct was integrated into the genome of the promacrophage cells and that CAT activity was increased as compared to non-genomic CAT constructs (see column 3, lines 3-30, for example).

Ivarie et al teach that the avian oviduct has potential as a bioreactor for inexpensive, simple production of large amounts of protein. Ivarie et al teach that tubular gland cells secrete egg white proteins such as lysozyme (column 1, lines 35-49 and 57-62, for example). Ivarie et al teach method of expressing exogenous proteins in an avian oviduct tubular gland cells so that the protein of interest is expressed in the

tubular gland cell of the oviduct and secreted into the lumen for deposit onto egg yolk (see column 4, lines 59-67 and column 6, lines 47-67). Ivarie et al teach the use of a vector containing a promoter sequence and an operably linked coding sequence so that the promoter can effect expression of the nucleic acid into the tubular gland cells. Ivarie et al disclose that the vectors integrated into the genome can contain promoters derived from the lysozyme gene and can be inserted into the avian genome (see column 5, lines 5-15 and column 6, lines 1-6, for example). Ivarie et al teach that the vectors contain a coding sequence and a magnum specific promoter, such as a lysozyme promoter, in operational and positional relationship to express the coding sequence in the tubular gland cell of the magnum of an oviduct.

It would have been obvious to one of ordinary skill in the art to combine the plasmid construct comprising a lysozyme gene enhancer and promoter comprising a 5' matrix attachment region operably linked to a CAT reporter gene and including a SV40 polyadenylation sequence as taught by Phi-Van et al (1996) with the method of chromosomal integration taught by Sippel and Stief, because Sippel and Stief teach that CAT expression is increased when a construct containing lysozyme matrix attachment regions linked to a CAT gene is integrated into a cellular genome.

It would also be obvious to the skilled artisan to chromosomally integrate the construct the plasmid construct comprising a lysozyme gene enhancer and promoter comprising a 5' matrix attachment region operably linked to a CAT reporter gene into a tubular gland cell because Ivarie et al teach that expression of exogenous genes under the direction of a lysozyme promoter is convenient when the expression vector is

incorporated into an avian tubular gland cell for production. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising a chicken lysozyme gene transcription enhancer region and 5' matrix attachment region into the tubular gland cell genome since this has worked previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186-187, 195-196, 207-208 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over von Kries et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and further in view of Ivarie et al (U.S Patent 6,730,822). This is a NEW rejection..

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject

matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell containing an expression vector as described above and containing an intrinsically curved region of DNA. Applicant further claims a tubular gland cell containing a DNA molecule comprising a gene controlling region encoding a heterogeneous polypeptide wherein the heterologous polypeptide is a protein of pharmaceutical interest.

von Kries et al teach a nucleotide sequence (GenBank Accession No. X52989) from the chicken lysozyme gene 5' matrix attachment region that is 98% identical to nucleotides 2011-2671 of SEQ ID NO:67 and strongly curved. von Kries et al teach that an isolated DNA sequence was cloned into a plasmid vector for sequencing (see page 3881, right column, 1st paragraph and page 3882, left column, 2nd paragraph, for example), which reads on an isolated DNA molecule and an expression vector comprising a nucleotide sequence or an **intrinsically curved region** of DNA that hybridizes to the nucleotide sequence of SEQ ID NO:67.

von Kries et al do not teach that the expression vector comprising an **intrinsically curved** lysozyme gene 5' matrix attachment region is integrated into a cellular genome. von Kries et al do not teach a tubular gland cell containing the expression vector comprising an intrinsically curved lysozyme gene 5' matrix attachment region.

The teachings of Sippel and Stief have been described herein above. The teaching of the BioTech Life Science Dictionary regarding CAT has been discussed above.

Ivarie et al teach that the avian oviduct has potential as a bioreactor for inexpensive, simple production of large amounts of protein. Ivarie et al teach that tubular gland cells secrete egg white proteins such as lysozyme (column 1, lines 35-49 and 57-62, for example). Ivarie et al teach method of expressing exogenous proteins in an avian oviduct tubular gland cells so that the protein of interest is expressed in the tubular gland cell of the oviduct and secreted into the lumen for deposit onto egg yolk (see column 4, lines 59-67 and column 6, lines 47-67). Ivarie et al teach the use of a vector containing a promoter sequence and an operably linked coding sequence so that the promoter can effect expression of the nucleic acid into the tubular gland cells. Ivarie et al disclose that the vectors integrated into the genome can contain promoters derived from the lysozyme gene and can be inserted into the avian genome (see column 5, lines 5-15 and column 6, lines 1-6, for example). Ivarie et al teach that the vectors contain a coding sequence and a magnum specific promoter, such as a lysozyme promoter, in

operational and positional relationship to express the coding sequence in the tubular gland cell of the magnum of an oviduct.

It would have been obvious to one of ordinary skill in the art to combine the plasmid construct comprising a **curved** lysozyme gene 5' matrix attachment region as taught by von Kries et al with the chromosomally integrated expression plasmid for CAT taught by Sippel and Stief and integrate the construct into a tubular gland cell chromosome because Sippel and Stief teach that gene expression is increased when a construct containing lysozyme matrix attachment regions linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a promoter such as lysozyme is convenient for protein production when the expression vector is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme matrix attachment promoter elements including intrinsically curved regions by integrating the constructs into host cell genomes and being able to inexpensively produce large amounts of a protein of interest in tubular gland cells as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating a plasmid expression vector containing a curved lysozyme gene 5' matrix attachment region into a tubular gland cell genome and expressing CAT since this has worked previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be

considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186, 188, 195-196, 207, 209 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over Grewal et al (of record) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and in view of Sippel and Stief (U.S. Patent 5,731,178) and further in view of Ivarie et al (U.S. Patent 6,730,822). This is a NEW rejection..

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell containing an expression vector as described above, containing a transcription enhancer. Applicant further claims a tubular gland cell containing a DNA molecule comprising a gene controlling region encoding a heterogeneous polypeptide wherein the polypeptide is a protein of pharmaceutical interest

Grewal et al teach a nucleotide sequence from the chicken lysozyme gene enhancer region that is located 6.1 kb upstream of the lysozyme transcription initiation site and which is 100% identical to nucleotides 5848-5934 of SEQ ID NO:67 (see page 2344, Figure 4 in particular). The -6.1kb enhancer element was isolated and cloned into a plasmid comprising a reporter gene such as CAT (see page 2340, left column). Grewal et al teach that the expression vector was transfected into several cell types and that the –6.1 kb lysozyme gene enhancer plays a central role in the activation of lysozyme expression (see page 2340, right column, last paragraph and page 2347, right column, for example).

Grewal et al does not teach that the expression vector comprising a -6.1kb enhancer element was integrated into a cellular genome. Grewal et al does not teach a tubular gland cell comprising an expression vector comprising a transcription enhancer element or a DNA molecule comprising a transcription enhancer element and a sequence encoding a heterologous polypeptide.

The teachings of Sippel and Stief have been described herein above. The teaching of the BioTech Life Science Dictionary regarding CAT has also been discussed above.

Ivarie et al teach that the avian oviduct has potential as a bioreactor for inexpensive, simple production of large amounts of protein. Ivarie et al teach that tubular gland cells secrete egg white proteins such as lysozyme (column 1, lines 35-49 and 57-62, for example). Ivarie et al teach method of expressing exogenous proteins in an avian oviduct tubular gland cells so that the protein of interest is expressed in the tubular gland cell of the oviduct and secreted into the lumen for deposit onto egg yolk (see column 4, lines 59-67 and column 6, lines 47-67). Ivarie et al teach the use of a vector containing a promoter sequence and an operably linked coding sequence so that the promoter can effect expression of the nucleic acid into the tubular gland cells. Ivarie et al disclose that the vectors integrated into the genome can contain promoters derived from the lysozyme gene and can be inserted into the avian genome (see column 5, lines 5-15 and column 6, lines 1-6, for example). Ivarie et al teach that the vectors contain a coding sequence and a magnum specific promoter, such as a lysozyme promoter, in operational and positional relationship to express the coding sequence in the tubular gland cell of the magnum of an oviduct.

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme enhancer as taught by Grewal et al with the method of chromosomal integration taught by Sippel and Stief and chromosomally integrate the construct into a tubular gland cell, because Sippel and Stief teach that CAT expression is increased when a construct containing lysozyme matrix attachment regions and **enhancers** linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a promoter

such as lysozyme is convenient for protein production when the expression vector is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and transcription enhancer into the tubular gland cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186, 192, 196, 207, 213 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over Renkawitz et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and further in view of Ivarie et al. This is a NEW rejection..

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an

invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell comprising the expression vector described *supra* integrated into a cellular genome, or a DNA molecule comprising the described expression vector wherein they comprise a **proximal lysozyme promoter** and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Renkawitz et al teach a nucleotide sequence (GenBank Accession No. M12532) from the chicken lysozyme gene promoter region that is 100% identical to nucleotides 11563-11877 of SEQ ID NO:67 and is a **proximal lysozyme promoter**, which has been cloned into a recombinant plasmid and injected into chicken oviduct cells (see page 504, left column, Figure 1 and page 509, left column, 2nd and 3rd paragraph). Renkawitz et al teach that the promoter is linked to a gene for T antigen (i.e. a

heterologous polypeptide) (see page 504, left column, 2nd paragraph, bridging to right column, 1st paragraph).

Renkawitz et al do not teach that the plasmid comprising the proximal lysozyme promoter is integrated into the genome. Renkawitz et al do not teach a tubular gland cell comprising claimed expression vectors or DNA molecules.

The teachings of Sippel and Stief have been described above. The teaching of the BioTech Life Science Dictionary regarding CAT has also been discussed above.

Ivarie et al teach that the avian oviduct has potential as a bioreactor for inexpensive, simple production of large amounts of protein. Ivarie et al teach that tubular gland cells secrete egg white proteins such as lysozyme (column 1, lines 35-49 and 57-62, for example). Ivarie et al teach method of expressing exogenous proteins in an avian oviduct tubular gland cells so that the protein of interest is expressed in the tubular gland cell of the oviduct and secreted into the lumen for deposit onto egg yolk (see column 4, lines 59-67 and column 6, lines 47-67). Ivarie et al teach the use of a vector containing a promoter sequence and an operably linked coding sequence so that the promoter can effect expression of the nucleic acid into the tubular gland cells. Ivarie et al disclose that the vectors integrated into the genome can contain promoters derived from the lysozyme gene and can be inserted into the avian genome (see column 5, lines 5-15 and column 6, lines 1-6, for example). Ivarie et al teach that the vectors contain a coding sequence and a magnum specific promoter, such as a lysozyme promoter, in operational and positional relationship to express the coding sequence in the tubular gland cell of an oviduct.

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme proximal promoter as taught by Renkawitz et al with the method of chromosomal integration taught by Sippel and Stief and integrate the construct into a tubular gland cell, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a lysozyme promoter for protein production is convenient when the expression vector is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit of being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme proximal promoters and transcription enhancers into the tubular gland cell genome since this has worked previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186, 188-189, 195-196, 207, 209-210 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over Steiner et al (of record) as evidenced by the online

BioTech Life Science Dictionary (CAT entry) in view of Sippel and Stief (U.S. Patent 5,731,178) and further in view of Ivarie et al. This is a NEW rejection.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell comprising expression vector described *supra* and a DNA molecule integrated into a cellular genome, wherein it comprises a transcription enhancer, or wherein it comprises a negative regulatory element and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Steiner et al teach a nucleotide sequence (E-0.2kb) (GenBank Accession No. X05461) from the chicken lysozyme gene transcription enhancer region that is 98% identical to nucleotides 9160-9329 of SEQ ID NO:67 (see page 4174, Figure 8). Steiner et al also teach a nucleotide sequence (GenBank Accession No. X05463) from the chicken lysozyme gene transcription enhancer region that is 98% identical to nucleotides 9325-9626 of SEQ ID NO:67 and a negative regulatory element (see page 4174, Figure 8). Steiner et al teach that the transcription enhancer region and negative regulatory element were isolated and cloned into plasmids containing the CAT gene (see page 4164, for example). Steiner et al teach that the plasmids were transfected into chicken fibroblast cells for CAT expression assays (see page 4166, last paragraph; page 4166, 2nd and 3rd paragraph, for example).

Steiner et al do not teach that the expression vector is integrated into a cellular genome. Steiner et al do not teach a tubular gland cell comprising the claimed expression vector or DNA molecules comprising a **negative regulatory element**.

The teachings of Sippel and Stief have been discussed herein above. The teaching of the BioTech Life Science Dictionary regarding CAT has also been discussed above. The teachings of Ivarie et al have been discussed herein above.

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme transcription **enhancer and negative regulatory element** driving CAT expression as taught by Steiner et al with the method of chromosomal integration taught by Sippel and Stief and integrate the construct into a tubular gland cell, because Sippel and Stief teach that CAT gene expression is

increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a lysozyme promoter is convenient for the production of the protein when the expression vector is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit being able to increase expression of genes of interest operably linked to lysozyme promoter elements such as a transcription enhancer and negative regulatory element by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising a lysozyme transcription enhancer and negative regulatory element into the tubular gland cell genome since this has worked previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186, 190, 195-196, 207, 211 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over Hecht et al (of record) as evidenced by the online BioTech Life Science Dictionary (CAT entry) in view of Sippel and Stief (U.S. Patent 5,731,178) and further in view of Ivarie et al. This is a NEW rejection..

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell comprising expression vector or a DNA molecule described *supra*, wherein they comprise at least one **hormone responsive element** and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Hecht et al teach a nucleotide sequence (GenBank Accession No. X12509) from the chicken lysozyme gene transcription enhancer region that is 99% identical to

nucleotides 9621-9666, 9680-10060 of SEQ ID NO:67 (see page 2070, Figure 6). Hecht et al teaches a DNA sequence which stimulates transcription in a **hormone dependent manner** which has been operably linked to a CAT gene in a vector for transfection into a hormone receptor containing cell line (see page 2063, right column, 2nd and 3rd paragraph, for example). The expression vector comprising at least one hormone responsive element was transfected into a human carcinoma line (see page 2071, right column, last paragraph, for example).

Hecht et al does not teach that the expression vector is integrated into a genome. Hecht et al do not teach a tubular gland cell comprising the claimed expression vector or DNA molecule.

The teachings of Sippel and Stief have been discussed above. The teaching of the BioTech Life Science Dictionary regarding CAT has also been discussed above. The teachings of Ivarie et al have been discussed above.

It would have been obvious to one of ordinary skill in the art to combine the expression construct comprising a lysozyme gene transcription enhancer region comprising a **hormone response element** to drive CAT expression as taught by Steiner et al with the method of chromosomal integration taught by Sippel and Stief and integrate the construct into a tubular gland cell genome, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a lysozyme promoter is convenient for protein production when the expression vector

is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit of being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest in tubular gland cells as suggested by Sippel and Stief and exemplified by Ivarie et al.. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising lysozyme promoters and enhancers such as a hormone response element into the tubular gland genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 186, 191, 195-196, 207, 212 and 216 are rejected under 35 U.S.C. 103(a) as being obvious over Stumph et al (of record) in view of Sippel and Stief (U.S. Patent 5,731,178) as evidenced by the online BioTech Life Science Dictionary (CAT entry) and further in view of Ivarie et al. This is a NEW rejection.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an

invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

Applicant claims a tubular gland cell comprising an expression vector (described above) or a DNA molecule integrated into a cellular genome, wherein they comprise an avian CR1 repeat element and wherein the gene expression controlling region is operably linked to a nucleic acid molecule encoding a heterologous polypeptide, wherein the polypeptide is a protein of pharmaceutical interest.

Stumph et al teach a nucleotide sequence (GenBank Accession No. K02907) that is a **CR1 chicken enhancer repeat** element that is 87% identical to nucleotides 10926-11193 of SEQ ID NO:67(see page 6669, Figure 2, for example). Stumph et al teach that CR1 sequences were cloned for sequencing into a plasmid vector (see page 6667, right column, 2nd and 3rd paragraph, for example).

Stumph et al does not teach an expression vector integrated into a cellular genome. Stumph et al do not teach a tubular gland cell comprising the claimed expression vector or DNA molecule.

The teachings of Sippel and Stief have been discussed herein above. The teaching of the BioTech Life Science Dictionary regarding CAT has also been discussed above. The teachings of Ivarie et al have been discussed herein above.

It would have been obvious to one of ordinary skill in the art to combine a CR1 chicken enhancer repeat element to drive gene expression as taught by Steiner et al with the expression vector and method of chromosomal integration taught by Sippel and Stief and integrate the construct into a tubular gland cell, because Sippel and Stief teach that CAT gene expression is increased when a construct containing lysozyme matrix attachment regions and enhancers linked to a CAT gene is integrated into a cellular genome and Ivarie et al teach that expression of exogenous genes under the direction of a lysozyme promoter is convenient for production of protein when the expression vector is incorporated into an avian tubular gland cell. The motivation to do so is the expected benefit as exemplified by Sippel and Stief being able to increase expression of genes of interest operably linked to lysozyme promoter elements by integrating the constructs into tubular gland cell genomes and being able to inexpensively produce large amounts of a protein of interest as suggested by Sippel and Stief and exemplified by Ivarie et al. There is reasonable expectation of success in integrating the lysozyme CAT construct comprising an avian CR1 repeat element into the tubular gland cell genome since this has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan

would have had a reasonable expectation of success in practicing the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura McGillem, PhD
6/16/2006

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